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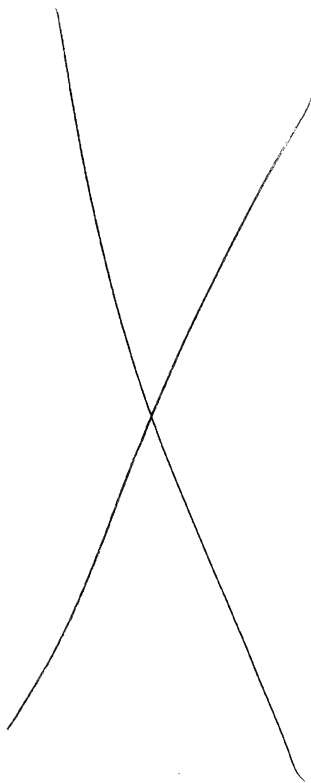
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9800445.0

3. Full name, address and postcode of the or of each applicant  
(underline all surnames) IMPERIAL COLLEGE OF SCIENCE,  
TECHNOLOGY & MEDICINE,  
SHERFIELD BUILDING,  
EXHIBITION ROAD,  
LONDON,  
SW7 2AZ.

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation BRITISH  
04033452004

4. Title of the invention METHOD FOR DESENSITISATION

5. Name of your agent (if you have one) D YOUNG & CO  
  
"Address for service" in the United Kingdom to which all correspondence should be sent  
(including the postcode) 21 NEW FETTER LANE  
LONDON  
EC4A 1DA  
  
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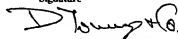
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## METHOD FOR DESENSITISATION

The present invention relates to a method for desensitising patients who are hypersensitive to particular allergens. Moreover, the invention relates to immunological vaccines which may be used to prevent and/or treat conditions involving hypersensitivity to allergens.

The ability of the immune system to elicit a response to a particular molecule depends critically upon its ability to recognise the presence of an antigen. Classically, the term antigen has been associated with the ability of a molecule to be an antibody generator *via* induction of B-cells. It is now known however that T cells also possess the ability to recognise antigens. T-cell antigen recognition requires antigen presenting cells (APCs) to present antigen fragments (peptides) on their cell surface in association with molecules of the major histocompatibility complex (MHC). T cells use their antigen specific T-cell receptors (TCRs) to recognise the antigen fragments presented by the APC. Such recognition acts as a trigger to the immune system to generate a range of responses to eradicate the antigen which has been recognised.

T lymphocytes have been implicated in the pathogenesis of a wide variety of diseases involving immune recognition of antigens derived both from the internal (host) and external environments. Autoimmune diseases such as autoimmune thyroiditis, rheumatoid arthritis and lupus erythematosus arise from the recognition by the immune system of host, or self antigens.

Recognition of external antigens by the immune system of an organism, such as man, can in some cases result in diseases, known as atopic conditions. An example of the latter are the allergic diseases including asthma, atopic dermatitis and allergic rhinitis. In this group of diseases, B lymphocytes generate antibodies of the IgE class (in humans) which bind externally derived antigens, which are referred to in this context as allergens, since these molecules elicit an allergic response. Production of allergen-specific IgE is dependent upon T lymphocytes which are also activated by (are specific

for) the allergen. Allergen-specific IgE antibodies bind to the surface of cells such as basophils and mast cells by virtue of the expression by these cells of surface receptors for IgE. Crosslinking of surface bound IgE molecules by allergen results in degranulation of these effector cells causing release of inflammatory mediators such as histamine, 5-hydroxytryptamine and lipid mediators such as the sulphidoleukotrienes. In addition to IgE-dependent events, certain allergic diseases such as asthma are characterised by IgE-independent events. It has been demonstrated that the induction of the late phase reaction is an IgE-independent event which is dependent upon the activation of allergen-specific T lymphocytes.

Allergic IgE-mediated diseases are currently treated by desensitisation procedures that involve the periodic injection of allergen components or extracts. Desensitisation treatments may induce an IgG response that competes with IgE for allergen, or they may induce specific suppressor T cells that block the synthesis of IgE directed against allergen. This form of treatment is not always effective and poses the risk of provoking serious side effects, particularly general anaphylactic shock. This can be fatal unless recognised immediately and treated with adrenaline. A therapeutic treatment that would decrease or eliminate the unwanted allergic-immune response to a particular allergen, without altering the immune reactivity to other foreign antigens or triggering an allergic response itself would be of great benefit to allergic individuals.

Asthma can be provoked by inhalation of allergen in the clinical laboratory under controlled conditions. The response is characterised by an early asthmatic reaction (EAR) followed by a delayed-in-time late asthmatic reaction (LAR) (See *Allergy and Allergic Diseases*, A.B. Kay (Ed.), Blackwell Science, pp. 1113 to 1130, 1997.). The EAR occurs within minutes of exposure to allergen, is maximal between 10 and 15 min and usually returns to near baseline by 1 hour. It is generally accepted that the EAR is dependent on the IgE-mediated release of mast cell-derived mediators such as histamine and leukotrienes. In contrast the LAR reaches a maximum at 6-9 hours and is believed to represent, at least in part, the inflammatory component of the asthmatic response and in this sense has served as a useful model of chronic asthma.

The late asthmatic response is typical of responses to allergic stimuli collectively known as late phase responses (LPR). LPR is seen particularly in the skin and the nose following intracutaneous or intranasal administration of allergens.

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Using cat allergic individuals (rhinitic and asthmatic), Norman *et al* (Am. J. Respir. Crit. Care Med., (1996) 154:1623-8) attempted to induce the counterpart of murine experimental T cell tolerance by subcutaneous injection of "T cell reactive peptides" (termed IPC1 and IPC2) in humans. Peptides were designed on the basis of patterns of  
10 epitope recognition of short overlapping peptides by Fel d 1 reactive T cell lines. It was found that peptides derived from chain 1 gave greater proliferative responses than chain 2, with the majority of activity being associated in the N terminal region of chain 1. IPC 1 and IPC 2 were considerably longer (27 amino acids each) than previously defined T-cell epitopes. This may have been partly responsible for immediate  
15 (presumed IgE-mediated) reactions in some patients following administration (Norman *et al.*, Op. Cit.). Large peptide doses (4 x 750 µg) were required to achieve minimal clinical efficacy. The choice of peptides for therapy was based upon reactivity of secondary T-cell lines derived from a large number of cat-allergic individuals and did not take into account primary T-cell reactivity (i.e. *ex vivo*), which may be more  
20 sensitive, or MHC class II haplotype.

Norman *et al* reported a number of adverse hypersensitivity reactions including respiratory, and other allergic, symptoms. As stated, some had a rapid time of onset i.e. with 10 minutes whereas others were not observed until several hours after IPC  
25 1/IPC2 administration (although there was no local redness or swelling at the site of injection). These results have been interpreted as indicating the unsuitability of the peptides for immunotherapy, the production of a LPR being considered to be undesirable (Wheeler and Drachenberg, (1997) Allergy 53:602-612).

WO 92/11859 describes a method of reducing immune response to an allergen in which a non-allergen derived, non-stimulating peptide which binds to specific MHC class II molecules of APCs is used to inhibit T-cell response to particular allergens.

- 5 WO 91/06571 purports to disclose peptides derived from human T-cell reactive feline protein which can be used in the diagnosis, treatment or prevention of cat allergy.

WO 94/24281 relates to peptides and modified peptides of the major house dust mite allergens. The modified peptides have the intent of reducing the level of undesirable  
10 side effects associated with desensitising therapies.

### Summary of the Invention

We have observed that peptide allergens used in immunotherapy associate with  
15 particular MHC types in patients. Moreover, successful desensitisation of patients is achieved where a patient exhibits an initial LPR to the peptide allergen.

Accordingly, in a first aspect the invention provides a method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an allergen capable  
20 of eliciting an allergic response in the patient, comprising the steps of:

- a) administering a candidate peptide to a patient and determining whether the peptide induces a late phase response; and
- 25 b) selecting a peptide capable of inducing a late-phase response as an immunotherapeutic agent.

In a second aspect, the invention provides a method for testing for candidate peptides for selection according to the first aspect of the invention, comprising the steps of:



- a) assaying a peptide or peptides in a T-cell proliferation assay and selecting peptides capable of inducing proliferation in the patient's T-cells;
- b) tissue-typing the patient to determine MHC type;
- 5 c) determining the MHC molecule(s) bound by each candidate peptide; and
- d) selecting a peptide or peptides satisfying part (a) above and capable of binding to an MHC type possessed by a patient, for use as a candidate peptide in a method
- 10 according to the first aspect of the invention.

In a third aspect, the invention provides a method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an antigen comprising the steps of:

- 15 a) tissue-typing the patient to determine MHC type; and
- b) selecting, from a database of peptides which are known to bind to particular MHC molecules and induce a late phase response in a patient possessing such MHC
- 20 molecules, one or more peptides capable of binding to the MHC molecules possessed by the patient.

In a fourth aspect, the invention provides a database of peptides characterised according to the first and second aspects of the invention.

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### Detailed Description of the Invention

TCRs are highly variable in their specificity. Variability is generated, as with antibody molecules, through gene recombination events within the cell. TCRs recognise antigen

30 in the form of short peptides bound to molecules encoded by the genes of the Major Histocompatibility Complex (MHC). These gene products are the same molecules that

- give rise to "tissue types" used in transplantation and are also referred to as Human Leukocyte Antigen molecules (HLAs) which terms may be used interchangeably within this document. Individual MHC molecules possess peptide binding grooves which, due to their shape and charge are only capable of binding a limited group of peptides. The
- 5 peptides bound by one MHC molecule may not necessarily be bound by other MHC molecules and thus, a "restriction" in peptide/MHC binding exists. As used herein the term "allergen peptide-binding MHC" will be used to mean the MHC molecule(s) that bind the said allergen or allergen-derived peptide.
- 10 When a protein molecule such as an antigen or allergen is taken up by antigen presenting cells such as B lymphocytes, dendritic cells, monocytes and macrophages, the molecule is enzymatically degraded within the cell. The process of degradation gives rise to peptide fragments of the molecule which, if they are of the appropriate size, charge and shape, may then bind within the peptide binding groove of certain
- 15 MHC molecules and be subsequently displayed upon the surface of antigen presenting cells. If the peptide/MHC complexes are present upon the antigen presenting cell surface in sufficient numbers they may then activate T cells which bear the appropriate peptide/MHC-specific T cell receptors.
- 20 Due to the polymorphic nature of the MHC, individuals in an outbred population such as man will express different combinations of MHC molecules on their cell surfaces. Since different MHC molecules can bind different peptides from the same molecule based on the size, charge and shape of the peptide, different individuals will display a different repertoire of peptides bound to their MHC molecules.
- 25 Identification of universal MHC-binding peptide epitopes in an outbred population such as man is more difficult than in inbred animals. On the basis of differential MHC expression between individuals and the inherent differences in peptide binding and presentation which this brings, it is unlikely that a single peptide can be identified
- 30 which will be of use for desensitisation therapy in man for most diseases unless the association of a particular MHC molecule with that disease is very strong. For

example, the HLA-B27 molecule has been shown to have a close relationship with ankylosing spondylitis, where approximately 90% of sufferers express HLA-B27. For some autoimmune diseases, certain disease HLA associations have been demonstrated e.g. HLA-DR4 and rheumatoid arthritis, but these associations are much weaker than for ankylosing spondylitis.

In allergic diseases, associations are even weaker if demonstrated at all. For this reason, it is unlikely that therapies centred around a single peptide (even an immunodominant one) or small numbers of peptides will be optimally effective as desensitisation therapies. The conclusion drawn in the art where MHC binding allergen epitopes have been identified is that even if an immunodominant epitope is identified, it would appear that it is required to react with a variety of restricted MHCs to be of therapeutic value (see Van Neervan RJJ et al., J Immunol (1994) 4203-4210, Higgins JA et al., J Allerg Clin Immunol (1994) 891-899).

As set forth herein, it has now been observed that a patient may be desensitised to a particular allergen by the administration of a peptide that is able to bind to at least one MHC molecule of said patient. According to the present invention, therefore, the concept of "universal" desensitising peptides is rejected in favour of a selective approach based on tissue type.

It can be hypothesised that eosinophil-dependent mucosal tissue damage, including LPR, is under T-cell control. For example, by *in situ* hybridisation the numbers of mRNA positive cells for the Th2-type (IL-4 and IL-5) and eosinophil-active cytokines (IL-3, IL-5 and GM-CSF) were shown to be elevated in asthmatics both at baseline (Robinson *et al.*, (1992) N Engl J Med 326: 298-304) and following allergen-induced LAR (Bentley *et al.*, (1993) Am J Respir Cell Mol Biol 8:35-42). Furthermore IL-4 and IL-5 mRNA co-localised largely to CD4<sup>+</sup> T cells (Ying *et al.*, (1997) J Immunol 158:3539-3544). A T cell component of the LAR is also suggested by the observation that cyclosporin A attenuated the LAR, but not the EAR, provoked by allergen inhalation (Sihra *et al.*, (1997) Thorax 52:447-452). Furthermore a single infusion of

anti-CD4 produced significant improvement in lung function in chronic corticosteroid-dependent asthmatics. However it has been difficult to determine whether T cell activation, as an initiating event, leads directly to airway narrowing in asthmatic patients and therefore an asthmatic response.

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As described herein, it has now been shown that T cells can be selectively activated, and thus rendered unresponsive. Moreover the anergising or elimination of these T-cells leads to desensitisation of the patient for a particular antigen. The desensitisation manifests itself as a reduction in response to an allergen or allergen-derived peptide, or preferably an elimination of such a response, on second and further administrations of the allergen or allergen-derived peptide. The second administration may be made after a suitable period of time has elapsed to allow desensitisation to occur; this is preferably any period between one day and several weeks. An interval of around two weeks is preferred.

15

Based on these results, the invention provides a method for desensitising a patient to an allergen which comprises the administration to the patient of a peptide specifically selected to induce LPR and subsequent desensitisation in the patient. The peptides for desensitisation may be selected according to whether they induce LPR.

20

LPR is defined as set forth in *Allergy and Allergic Diseases*, A.B. Kay (Ed.), Blackwell Science, pp. 1113 to 1130, 1997 and includes asthmatic, cutaneous and nasal late phase responses. Preferably, the invention relates to late asthmatic responses (LAR).

25

Preferably, the peptides are derivatives of the allergen itself, and retain at least one common antigenic determinant of the allergen. "Common antigenic determinant" means that the derivative in question retains at least one antigenic function of the allergen. Antigenic functions include possession of an epitope or antigenic site that is capable of binding to TCRs which recognise the allergen or fragments thereof. Thus the peptides provided by the present invention include splice variants encoded by mRNA generated by alternative splicing of a primary transcript encoding the allergen, amino acid

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mutants, glycosylation variants and other covalent derivatives of the allergen which retain at least an MHC-binding property of the allergen. Exemplary derivatives include molecules wherein the peptide of the invention is covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid. Further included are naturally occurring variants of the allergen found in a particular species, preferably a mammal. Such a variant may be encoded by a related gene of the same gene family, by an allelic variant of a particular gene, or represent an alternative splicing variant of the allergen gene.

- 10 Derivatives of the allergen also comprise mutants thereof, which may contain amino acid deletions, additions or substitutions, subject to the requirement to maintain at least one feature characteristic of the allergen. Thus, conservative amino acid substitutions may be made to peptides according to the invention substantially without altering the nature of the allergen, as may truncations from the 5' or 3' ends. Deletions and  
15 substitutions may moreover be made to the fragments of the allergen comprised by the invention. Peptides may be produced from a DNA which has been subjected to *in vitro* mutagenesis resulting e.g. in an addition, exchange and/or deletion of one or more amino acids. Preferably, peptides are produced by peptide synthesis according to known techniques using commercially available peptide synthesisers. Mutations and/or  
20 truncations may thus be made by changing the amino acid sequence during the synthesis procedure.

- Suitable variants capable of binding to TCRs may be derived empirically or selected according to known criteria. Within a single peptide there are certain residues which  
25 contribute to binding within the MHC antigen binding groove and other residues which interact with hypervariable regions of the T cell receptor (Allen *et al.*, Nature 1987:327:713-5). Within the residues contributing to T cell receptor interaction, a hierarchy has been demonstrated which pertains to dependency of T cell activation upon substitution of a given peptide residue. Using peptides which have had one or more T  
30 cell receptor contact residues substituted with a different amino acid, several groups have demonstrated profound effects upon the process of T cell activation. In 1991

Evavold and Allen (Evavold and Allen, Nature 1991;252:1308-10) demonstrated the dissociation of T cell proliferation and cytokine production. In this *in vitro* model, a T cell clone specific for residues 64-76 of haemoglobin (in the context of I-E<sup>k</sup>), was challenged with a peptide analogue in which a conservative substitution of aspartic acid for glutamic acid had been made. This substitution did not significantly interfere with the capacity of the analogue to bind to I-E<sup>k</sup>. Following *in vitro* challenge of a T cell clone with this analogue, no proliferation was detected although IL-4 secretion was maintained, as was the capacity of the clone to help B cell responses. In a subsequent study the same group demonstrated the separation of T cell-mediated cytotoxicity from cytokine production. In this instance, the former remained unaltered while the latter was impaired. The efficacy of altered peptide ligands *in vivo* was initially demonstrated in a murine model of EAE by McDevitt and colleagues (Smilek *et al.*, Proc Natl Acad Sci USA 1991; 88:9633-9637). In this model EAE is induced by immunisation with the encephalitogenic peptide Ac1-11 of MBP. Substitution at position four (lysine) with an alanine residue generated a peptide which bound well to its restricting element (A $\alpha$ A $\beta$ ), but which was non-immunogenic in the susceptible PL/JxSJLF1 strain and which, furthermore prevented the onset of EAE when administered either before or after immunisation with the encephalitogenic peptide. Thus, residues can be identified in peptides which affect the ability of the peptides to induce various functions of T-cells.

Advantageously, peptides may be designed to favour T-cell proliferation and induction of desensitisation. Metzler and Wraith have demonstrated improved tolerogenic capacity of peptides in which substitutions increasing peptide-MHC affinity have been made (Metzler and Wraith, Int Immunol 1993;5:1159-65). The demonstration that an altered peptide ligand can cause long-term and profound anergy in cloned T cells (Sloan-Lancaster *et al.*, Nature 1993;363:156-9) is particularly relevant to the applications of such peptide analogues in immunotherapy for diseases such as autoimmunity and allergy, in addition to the induction of host/donor-specific tolerance in transplantation.

- Derivatives which retain common antigenic determinants are preferably fragments of the allergen. Fragments of the allergen comprise individual domains thereof, as well as smaller polypeptides derived from the domains. Preferably, smaller polypeptides derived from the allergen according to the invention define a single epitope of the allergen capable of binding a TCR. Fragments may in theory be almost any size, although smaller fragments are more likely to be restricted to a single MHC molecule and are thus preferred. Preferably, fragments will be between 5 and 50, preferably between 5 and 25, and advantageously about 17 amino acids in length.
- 10 Candidate peptides potentially capable of inducing LPR in a patient may be preselected in order to maximise the chances of identifying a therapeutically useful peptide in *in vivo* tests. The essential steps of this second aspect of the invention comprise the determination that the peptide is capable of causing T-cell proliferation when associated with an MHC molecule present in the patient to be treated. Thus, the selection
- 15 procedure can be broken down into three steps, performed either sequentially (in any order) or together:
- a) assaying a peptide or peptides in a T-cell proliferation assay and selecting peptides capable of inducing proliferation in a patient's T-cells;
  - 20 b) tissue-typing the patient to determine MHC type; and
  - c) determining the MHC molecule bound by each candidate peptide.
- 25 Steps (a) and (c), in particular, may be combined in a single T-cell proliferation assay. Preferably, the assay involves the use of cells transfected to express a particular MHC molecule, and the binding of the peptide to this MHC assessed by its ability to induce T-cell proliferation in the presence of the transfected cells alone.

Preferably, a peptide selected according to the above procedure is tested for its ability to induce LPR in a patient. If LPR is induced, repeated administration will result in desensitisation of the patient to the allergen from which the peptide is derived.

- 5 However, once a peptide has been determined to bind a particular MHC type and to be capable of inducing LPR when administered to a patient possessing that MHC type, it can be used to induce desensitisation to the relevant antigen in substantially any patient possessing the required MHC molecule. Therefore, peptides derived from particular allergens may be characterised according to their binding to particular MHC types and  
10 their ability to induce LPR, thus providing a database from which a suitable peptide may be selected for any given patient upon tissue typing of that patient.

Thus, antigen presenting cells may be isolated from a patient known to be sensitive to a particular allergen or allergens, and based on the peptide-binding MHC molecules  
15 displayed by said cells, a peptide may be selected for use in desensitising said patient by virtue of its ability to bind to at least one MHC molecule. The invention accordingly provides a method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an antigen comprising the steps of:

- 20 a) tissue-typing the patient to determine MHC type; and
- b) selecting, from a database of peptides which are known to bind to particular MHC molecules and induce a late phase response in a patient possessing such MHC molecules, one or more peptides capable of binding to the MHC molecules possessed  
25 by the patient.

For the avoidance of doubt, the patient referred to in part (b) above need not necessarily be the same patient undergoing treatment whom is tissue typed in part (a).

- 30 Allergens that may be amenable to desensitisation procedures as described herein include the peptides derived or chosen from the list comprising the allergens; Fel d 1



(the feline skin and salivary gland allergen of the domestic cat Felis domesticus - the amino acid sequence of which is disclosed in WO 91/06571), Der p I, Der p II, Der fI or Der fII (the major protein allergens from the house dust mite dermatophagoides - amino acid sequences disclosed in WO 94/24281).

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The invention is applicable substantially to any allergen, including allergens present in any of the following: grass, tree and weed (including ragweed) pollens; fungi and moulds; foods e.g. fish, shellfish, crab lobster, peanuts, nuts, wheat gluten, eggs and milk; stinging insects e.g. bee, wasp and hornet and the chironomidae (non-biting midges); spiders and mites, including the house dust mite; allergens found in the dander, urine, saliva, blood or other bodily fluid of mammals such as cat, dog, cows, pigs, sheep, horse, rabbit, rat, guinea pig, mouse and gerbil; airborne particulates in general; latex; biological detergent additives; drugs e.g. penicillins and other antibiotic and anaesthetic agents.

15

Where the allergen is an insect protein, the peptides may be selected from the group comprising: housefly, fruit fly, sheep blow fly, screw worm fly, grain weevil, silkworm, honeybee, non-biting midge larvae, bee moth larvae, mealworm, cockroach and larvae of Tenibrio molitor beetle. All these being insect allergens, they are of particular relevance to allergic problems arising in the workplace.

20

Where the allergen is the Fel d 1 allergen, useful peptides may preferably comprise a sequence as shown in any one of Sequence ID Nos. 1 to 3.

25

A database according to the invention includes information on the MHC molecule(s) bound by peptides in the database and optionally the ability of the peptides to induce a LPR in patients possessing such MHC molecule(s). Thus, the database allows a practitioner to select peptides capable or potentially capable of eliciting a LPR and therefore desensitisation in a particular patient on the basis of that patient's tissue type.

30

The invention moreover provides a peptide listed in a database according to the invention, for use in therapy. Preferably, such peptides are useful in methods for desensitising patients to allergens in accordance with the methods set forth herein.

- 5 The MHC molecules expressed on APCs which bind peptides derived from a specific allergen may be identified by methods known in the art, such as T cell proliferation studies with MHC blocking antibodies, and PCR techniques, for example techniques based on those of Olerup and Zetterquist, (1992) Tissue Antigens 29:225-235. Thus, antigen-presenting cells, expressing a variety of MHC molecules may be incubated with  
10 allergen and T cells and the latter observed for proliferation. Addition of antibodies to specific MHC classes may then be made in repeat incubations in order to identify the restricted MHC in respect of the allergen being tested. See Van Neerven RJJ et al., Immunol (1994) 82 351-356 and Yssel H et al., J Immunol (1992) 148 738-745).

- 15 Alternatively, cells presenting a single MHC type, for example cells such as fibroblast cells transfected with a gene encoding an MHC molecule, may be incubated with individual peptides for which T-cell clones or lines are known to be specific. Culturing of such T-cell clones or lines with peptide presented by the appropriate MHC molecule will lead to T-cell proliferation.

- 20 Preferred fibroblasts for use in this aspect of the invention include human or murine fibroblasts, particularly L-cells.

- The latter method may be used in a combinatorial approach, in which groups of  
25 peptides may be tested together and effective peptides identified by standard combinatorial techniques.

- Specific epitopes of the allergen or peptide derived therefrom that bind to at least one MHC molecule may then be identified by standard procedures and used in  
30 desensitisation procedures as described herein. Accordingly, the invention provides peptides when selected according to the foregoing aspects of the invention.

For example, when the allergen is a cat allergen such as the Fel d 1 protein, then the MHC molecule may include DR13 or DR1 class II MHC, and a peptide that binds to DR13 and/or DR1 or any of its sub-types that may be used in a desensitisation procedure is that shown in SEQ. ID No. 3.

The peptides identified in such a manner, and those of use in the methods of the present invention may be used in desensitisation procedures that typically involve sequential administration of said peptide. Generally, the first administration of the peptide will induce LPR. Subsequent administration will lead to desensitisation of the patient. For example, if the peptide is that of SEQ. ID No. 3 (a fragment of the Fel d 1 allergen), then upon first administration of this peptide a LPR will be observed. Subsequent administration of this peptide results in a weaker reaction or no reaction, the patient having been desensitised.

The invention also relates to the use of a peptide in desensitising a patient against an allergen, the peptide being identified by its capability to bind to at least one MHC molecule present in the patient and induce LPR in the patient.

Peptides may be administered to a patient singly or in combination. Thus, the database according to the invention may be used to prepare a designer vaccine which may be used to desensitise a patient to a chosen allergen, on the basis of the patient's MHC type. The MHC type can be correlated to the known MHC binding characteristics of the peptides listed in the database, and the appropriate peptides selected and combined to form a designer vaccine.

Whilst it is possible to design a vaccine which targets all or most of the epitopes on a particular antigen, this is unnecessary due to linked suppression of T-cells. Linked suppression is a phenomenon in which administration of a single epitope from a protein leads to the induction of a population of regulatory peptide-specific T lymphocytes which, by release of soluble factors such as TGF $\beta$  and/or IL-10, are able to suppress or

modify responses of non-tolerant T cells specific for other epitopes within the same protein and in some models epitopes derived from other proteins ("bystander suppression") (Davies *et al.*, J Immunol 1996;156:3602-7). In transplantation models, such regulatory T cells have been demonstrated to be capable of inducing a similar phenotype in naive T cells. This has given rise to the term "infectious tolerance" (Qin *et al.*, Science 1993;259:974-7) which may be a mechanism for effecting long-term hyporesponsiveness.

Linked suppression is thought to occur when peptide-specific regulatory T cells engage peptide/MHC complexes on the surface of the same or neighbouring APC as T cells specific for other epitopes. The latter may be responding to epitopes derived from the same molecule as the regulatory T cells or from a distinct molecule being processed by the same APC. This phenomenon allows desensitisation of patients to one or multiple allergens by the administration of a limited number of peptides.

Whilst it may be possible for the peptides according to the invention to be presented in raw form, it is preferable to present them as a pharmaceutical formulation. Thus, according to a further aspect, the present invention provides a pharmaceutical formulation comprising a peptide according to the invention together with one or more pharmaceutically acceptable carriers therefor and optionally one or more other therapeutic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral (particularly inhaled), parenteral (including subcutaneous, transdermal, intradermal, intramuscular and intravenous and rectal) administration, although the most suitable route may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association a compound of the present invention as herein defined or a pharmacologically acceptable

salt or solvate thereof ("active ingredient") with the carrier which constitutes one or more accessory ingredients.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Formulations for inhalation may be presented in any of the ways known to be effective e.g. metered dose inhalers.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example, water-for-injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol.

Preferred unit dosage formulations are those containing an effective dose, as hereinbelow recited, or an appropriate fraction thereof, of the active ingredient.

The compounds of the invention may typically be administered intranasally, by inhalation, orally or via injection at a dose of from 0.0001 to 1 mg/kg per dose.

Preferred are doses in the region of 10 to 150  $\mu\text{g}$  per human patient, advantageously about 80  $\mu\text{g}$ .

The invention is further described, for the purpose of illustration only, in the following examples, which refer to the figures.

Figure 1. The three peptides comprising FC1P (solid circles; 80 $\mu\text{g}$ ) or vehicle control (open circles) are injected intradermally at time zero on two separate days. Forced expiratory volume in 1 second (FEV1) is measured at intervals as a readout of lung function over a 24hr period. The use of rescue medication is indicated by arrows.

Figure 2. Repeated administration of FC1P leads to a reduced lung response. Three patient volunteers who develop a late asthmatic reaction following administration of FC1P (closed circles), are challenged again with the same dose after a period of at least 2 weeks. No significant fall in FEV1 is observed following the second challenge (closed triangles). Open circles indicate the control day. Arrows indicate the use of bronchodilators.

Figure 3. Murine L cells expressing two DR13 variants, DRB1\*1301 and 1302 are incubated overnight with each of the three FC1P peptides, or a control peptide, or medium alone. Cells are washed and incubated for one hour with a cytostatic agent to prevent proliferation in the subsequent assay. L cells are then incubated for 48 hours with T cells from a T cell line raised to whole cat dander (and including the Fel d 1 protein). Proliferation of the T cells is measured by their incorporation of the radiolabelled compound  $^3\text{H}$ -thymidine. T cells demonstrate a statistically significant response to the DR13 L cells and peptide FC1P3 (KALPVVLENARILNCV) but not to the other peptides/control.

Figure 4. Human fibroblasts expressing the DR1 allele DRB1\*0101 are incubated overnight with each of the three FC1P peptides, or medium alone, as described for Figure 3. T cells demonstrate a statistically significant response to the DR1 expressing

cells and peptide FC1P3 (KALPVVLENARILNCV) but not to the other peptides/control.

## EXAMPLES

### Experimental Techniques

#### *Primary Proliferation Assays.*

PBMCs are separated from whole blood by density gradient centrifugation according to standard methods. Cultures are established at  $2 \times 10^5$  cells per well in flat bottomed 96 well plates with 3 concentrations each individual peptide, or a optimum concentration of cat dander cat allergen extract, medium (negative control) or PPD (positive control). Cells are cultured for 8 days (cat dander) and 6 days (all others) and pulsed with  $1 \mu\text{Ci}$  tritiated thymidine. Cultures are harvested and counted after 8-16 hours.

#### *T Cell Clones*

PBMCs are cultured in 24 well plates with cat dander for 10 - 12 days, with the addition of approximately 10ng IL-2 on days 5 and 7, restimulated twice with irradiated autologous PBMCs and cat dander, and the line expanded with Phytohaemagglutinin (PHA) and IL-2. Clones are established by limiting dilution and will subsequently be frozen for use at a later stage to determine changes in cytokine secretion.

### Example 1

#### *Preparation Of Allergen Peptides*

The sequence of chain 1 of the cat allergen Fel d 1 is shown in SEQ. ID. No. 4; chain 2 is shown in SEQ. ID. No. 5. Multiple overlapping peptides are designed around this sequence, as well as that of chain 2 of Fel d 1, as shown in Table 1.

TABLE I

## Feld I Chain I

5	Native sequence	EICPAVKRDVDLFLTGTPDEYVEQYKALPVVLENARILKNCVDKMTTEEDKENALSLLDKIYTSPLC
	Peptide 1.1	EICPAVKRDVDLFLT
10	Peptide 1.2	LFLTGTPDEYVEQYQY
	Peptide 1.3	EQVAQYKALPVVLENA
	Peptide 1.4	KALPVVLENARILKNCV
15	Peptide 1.5	RILKNCVDKMTTEEDKE
	Peptide 1.6	KMTTEEDKENALSLLDK
20	Peptide 1.7	KENALSLLDKIYTSPL



21

Fel d I Chain 2

Native Sequence

5 VKMAETCPIFYDVFFAVANGNELLLKLSLTQVKNATEPERTAMKKIQDCYVENGILSRVLDGLVNTTISSSKDCMGEAVQNTVEDLKLNLTIGR

VKMAETCPIFYDVFFA Peptide 2.1

CPIFYDVFFAVANGNEL Peptide 2.2

10

GNELLKLSLTQVKNAT Peptide 2.3

LTKVNATEPERTAMKK Peptide 2.4

15

TAMKKIQDCYVENGIL Peptide 2.5

CYVENGILSRVLDGLV Peptide 2.6

SRVLDGLVNTTISSSK Peptide 2.7

20

ISSSKDCMGEAVQNTV Peptide 2.8

AVQNTVEDLKLNLTIGR Peptide 2.9

25

## Example 2

### *Observation Of LAR In Patients On Peptide Administration*

A single intradermal administration (80µg of each peptide) of a mixture containing three short peptides (SEQ. ID Nos. 1, 2 or 3) is given to 18 cat asthmatic individuals. 6 patients develop an isolated late asthmatic reaction as shown in Figure 1 wherein a greater than 20% fall in Forced Expiratory Volume in 1 second (FEV1 - a measure of lung function) is considered as a positive asthmatic effect. The results are shown in Figure 1 where the three peptides comprising FC1P (solid circles) or vehicle control (open circles) are injected intradermally at time zero on two separate days. FEV1 is measured at intervals as a readout of lung function over a 24hr period. The use of rescue medication is indicated by arrows.

This result demonstrates that peptides capable of causing a LPR can be derived from a common allergen such as cat dander and tested for LAR production in cat asthmatic patients.

Three patient volunteers who develop a late asthmatic reaction following administration of FC1P (closed circles), are challenged again with the same dose after a period of at least 2 weeks. No significant fall in FEV1 is observed following the second challenge (closed triangles). Open circles indicate the control day. Arrows indicate the use of bronchodilators. As shown in Figure 2, none of the three develop a late asthmatic reaction to the second peptide administration indicating that the immune response to this peptide has been downregulated.

## Example 3

### *Correlation between Tissue type and LAR*

The 18 patients observed in Example 2 are MHC-typed using PCR, based upon the method of Olerup and Zetterquist, (1992) Tissue Antigens 29:225-235. Four of the 6 reactors express HLA-DR13 (a closely related family of MHC molecules) compared to 1 out of 12 of the non-reactors. These results indicate that one of the three peptides

injected is capable of binding to a DR13 family member and thus stimulating peptide-specific T cells from the reactors.

5 In order to demonstrate that specific T cells have been activated, L cells which have been transfected with the human genes encoding two DR13 family members are obtained from Georgetown University Medical School, USA. Murine L cells expressing two DR13 variants, DRB1\*1301 and 1302 are incubated overnight with each of the three FC1P peptides, or a control peptide, or medium alone. Cells are washed and incubated for one hour with a cytostatic agent to prevent proliferation in the subsequent  
10 assay. L cells are then incubated for 48 hours with T cells from a T cell line raised from PBMCs isolated from a reactor patient as described above and stimulated weekly with whole cat dander (and including the Fel d 1 protein). Proliferation of the T cells is measured by their incorporation of the radiolabelled compound  $^3\text{H}$ -thymidine. T cells demonstrate a statistically significant response to the DR13 L cells and peptide FC1P3  
15 (SEQ. ID No 3) but not to the other peptides/control as shown in Figure 3.

A further experiment is performed with human fibroblasts expressing the DR1 variant DRB1\*0101. Cells are incubated overnight with each of the three FC1P peptides, or medium alone, washed, treated and incubated with T-cells as described above for the  
20 DR13 variants. T cells demonstrate a statistically significant response to the DR1 L cells and peptide FC1P3 (SEQ. ID No 3) but not to the other peptides/control as shown in Figure 4.

It is demonstrated that FC1P3 is capable of binding to both DR1 and DR13 MHC  
25 molecules and activating T cells, thereby inducing the isolated late asthmatic reaction shown in Figure 1. This results correlates extremely well with the tissue type data obtained from the patient population, wherein 4 out of six reactors are DR13 and two are DR1, compared with 1 out of 12 DR1 and 1 out of 12 DR13 non-reactors.

#### Example 4

##### *FCIP3 induces LAR and desensitisation in tissue-typed patients*

Patients are selected on the basis of being allergic to cat dander, as in the previous examples. T-cell lines are prepared from each patient as described above, and maintained with weekly stimulation with cat dander extract. The patients are tissue-typed, and patients possessing DR1 or DR13 variants selected.

In order to predict the ability of peptide FCIP3 to desensitise the patients against cat dander, T-cell proliferation assays are performed using T-cells isolated from the patients as described and human fibroblasts or murine L-cells transfected with DR1 or DR13 alleles in the presence of FCIP3 according to Example 3. The T-cells are observed to proliferate, by the incorporation of  $^3\text{H}$ -thymidine, indicating that T-cells isolated from DR13 and DR1 possessing patients are responsive to stimulation with the FCIP3 peptide.

FCIP3 peptide is injected into patients which are DR1 and/or DR13 positive and in respect of whom a positive result has been obtained in the T-cell proliferation assay. These patients experience a LAR response, as measured by a 20% or greater fall in FEV1.

Patients who develop a late asthmatic reaction following administration of FCIP3 are challenged again with the same dose after a period of 2 weeks. As in Example 2, no significant fall or a reduced fall in FEV1 is observed following the second challenge, indicating that the immune response to this peptide has been downregulated.

#### Example 5

##### *MHC restriction mapping of Fel d 1*

In order to prepare a database of Fel d 1 derived peptides characterised according to MHC type restriction, an *in vitro* study of MHC class II restriction mapping is performed using a panel of L cells, T-cell lines to whole cat allergen and the overlapping peptides from chain 1 and chain 2, as described in Example 1. T cell

lines with specificity for whole cat extract (which includes Fel d 1) are generated from the peripheral blood of subjects before peptide administration according to the procedures described above. Subjects are HLA-DR, DP and DQ typed, and, based on their expression, initially of DR alleles, transfected fibroblasts are selected to assay T-cell stimulation by each of the peptides.

Where the required HLA type clone is not available, MHC genes are cloned directly from the patient's cells by PCR amplification and cloning, as described above. Cloned genes are subsequently expressed in murine L-cells.

Cell lines (generous gifts from Prof. J.R. Lamb, University of Edinburgh, Prof. R.I. Lechler and Dr. G. Lombardi, ICSM, Hammersmith Hospital, Dr. C. Hurley and Dr. J.R. Richert, Georgetown University Medical Center, Washington, USA) expressing the appropriate restriction element are incubated with each individual Fel d 1 peptide as described in Example 3. Following incubation in the cytostatic agent mitomycin C to prevent L cell division, cells are extensively washed and incubated with the T cell line. Proliferative responses are measured after 48 hours by addition of tritiated thymidine to all cultures for 8-16 hours. Peptides eliciting a proliferative response from the T cell line are thus restricted by the HLA allele expressed by the chosen L cell line.

Administration of peptides obtained from the database to patients possessing the HLA type in respect of which a proliferative response is seen in the above assay in the majority of cases results in a LAR, as expected, which is followed by desensitisation of the patient to cat dander on subsequent administration of the peptides.

In this way an MHC class II restriction map of the Fel d 1 molecule is constructed such that the appropriate peptides for immunotherapy may subsequently be selected on an individual patient basis, solely by virtue of that subject's HLA type.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

5

## (i) APPLICANT:

(A) NAME: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND  
MEDICINE

(B) STREET: SHERFIELD BUILDING, EXHIBITION ROAD

10

(C) CITY: LONDON

(E) COUNTRY: GB

(F) POSTAL CODE (ZIP): SW7 2AZ

## (ii) TITLE OF INVENTION: METHOD FOR DESENSITISATION

15

## (iii) NUMBER OF SEQUENCES: 5

## (iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

20

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

25

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids

(B) TYPE: amino acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

35

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val Glu Gln Val Ala Gln  
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Glu Gln Val Ala Gln Tyr Lys Ala Leu Pro Val Val Leu Glu Asn Ala  
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

15

Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys Asn Cys

1

5

10

15

Val

20

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

25

(A) LENGTH: 70 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

35



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Glu Ile Cys Pro Ala Val Lys Arg Asp Val Asp Leu Phe Leu Thr Gly  
 1 5 10 15

Thr Pro Asp Glu Tyr Val Glu Gln Val Ala Gln Tyr Lys Ala Leu Pro  
 20 25 30

Val Val Leu Glu Asn Ala Arg Ile Leu Lys Asn Cys Val Asp Ala Lys  
 35 40 45

Met Thr Glu Glu Asp Lys Glu Asn Ala Leu Ser Leu Leu Asp Lys Ile  
 50 55 60

Tyr Thr Ser Pro Leu Cys  
 65 70

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 92 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Val Lys Met Ala Glu Thr Cys Pro Ile Phe Tyr Asp Val Phe Phe Ala  
 1 5 10 15

Val Ala Asn Gly Asn Glu Leu Leu Leu Lys Leu Ser Leu Thr Lys Val  
 20 25 30

5 Asn Ala Thr Glu Pro Glu Arg Thr Ala Met Lys Lys Ile Gln Asp Cys  
 35 40 45

Tyr Val Glu Asn Gly Leu Ile Ser Arg Val Leu Asp Gly Leu Val Met  
 50 55 60

10

Thr Thr Ile Ser Ser Ser Lys Asp Cys Met Gly Glu Ala Val Gln Asn  
 65 70 75 80

15

Thr Val Glu Asp Leu Lys Leu Asn Thr Leu Gly Arg  
 85 90

20

## CLAIMS

1. A method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an allergen capable of eliciting an allergic response in the patient, comprising the steps of:
  - a) administering a candidate peptide to a patient and determining whether the peptide induces a late phase response; and
  - b) selecting a peptide capable of inducing a late phase response as an immunotherapeutic agent.
2. A method for testing for candidate peptides for selection by a method according to claim 1, comprising the steps of:
  - a) assaying a peptide or peptides in a T-cell proliferation assay and selecting peptides capable of inducing proliferation in a patient's T-cells;
  - b) tissue-typing the patient to determine MHC type;
  - c) determining the MHC molecule bound by each candidate peptide; and
  - d) selecting a peptide or peptides satisfying part (a) above and capable of binding to an MHC type possessed by a patient, for use as a candidate peptide in a method according to claim 1.
3. A method according to claim 1 or claim 2, wherein the peptide is derived from the allergen.
4. A method according to claim 3, wherein the peptide is a fragment of the allergen.

5. A method according to claim 3 or claim 4, wherein the allergen is selected from the group consisting of: Fel d 1, Der p I, Der p II, Der fl or Der flI and allergens present in any of the following: grass, tree and weed (including ragweed) pollens; fungi  
5 and moulds; foods, stinging insects, the chironomidae (non-biting midges); spiders and mites, housefly, fruit fly, sheep blow fly, screw worm fly, grain weevil, silkworm, honeybee, non-biting midge larvae, bee moth larvae, mealworm, cockroach, larvae of Tenibrio molitor beetle, mammals such as cat, dog, horse, cow, pig, sheep, rabbit, rat, guinea pig, mice and gerbil; airborne particulates; latex; biological detergent additives  
10 and drugs.

6. A method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an antigen comprising the steps of:

- 15
- a) assaying a peptide or peptides in a T-cell proliferation assay and selecting peptides capable of inducing proliferation in a patient's T-cells;
  - b) tissue-typing the patient to determine MHC type;
  - 20 c) determining the MHC molecule bound by each candidate peptide;
  - d) administering a candidate peptide to a patient possessing the peptide-bound MHC molecule and determining whether the peptide induces a late phase response; and  
25 e) selecting a peptide capable of inducing a late phase response as an immunotherapeutic agent.

7. A method according to any preceding claim, wherein the MHC molecule is  
30 selected from HLA-DR, HLA-DP, HLA-DQ and subclasses thereof.

8. A peptide when selected according to the method of any one of claims 1 to 7.
9. Use of a peptide capable of eliciting a late phase reaction in a patient in the manufacture of a composition suitable for desensitising a patient to an allergen.
- 5
10. Use according to claim 9 wherein the allergen is cat dander and the peptide is KALPVVLENARILNCV (SEQ. ID. No. 3).
- 10
11. A database of peptides characterised according to their ability to bind an MHC molecule and induce a late phase response in a patient possessing the MHC molecule.
12. A peptide listed in a database according to claim 11, for use in therapy.
13. A method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an antigen comprising the steps of:
- 15
- a) tissue-typing the patient to determine MHC type; and
- b) selecting, from a database of peptides which are known to bind to particular MHC molecules and induce a late phase response in a patient possessing such MHC molecules, one or more peptides capable of binding to the MHC molecules possessed by the patient.
- 20

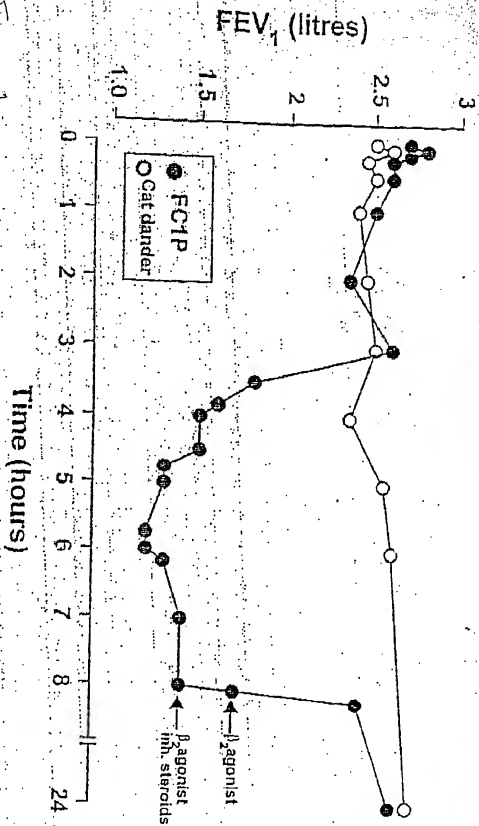
**ABSTRACT**

The invention relates to a method for desensitisation and provides a method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an allergen capable of eliciting an allergic response in the patient, comprising the steps of: (a) administering a candidate peptide to a patient and determining whether the peptide induces a late phase response; and (b) selecting a peptide capable of inducing a late phase response as an immunotherapeutic agent, as well as the use of a peptide capable of eliciting a late phase reaction in a patient in the manufacture of a composition suitable for desensitising a patient to an allergen.

15

20

Figure 1



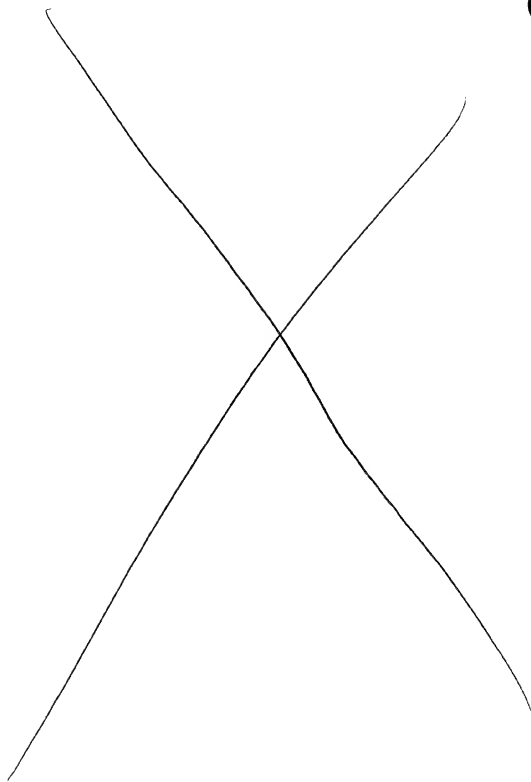
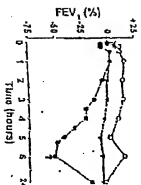


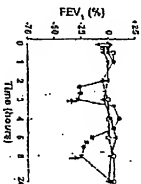


FIGURE 2

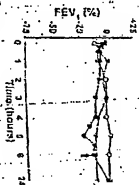
(Patient 1)



(Patient 2)



(Patient 3)



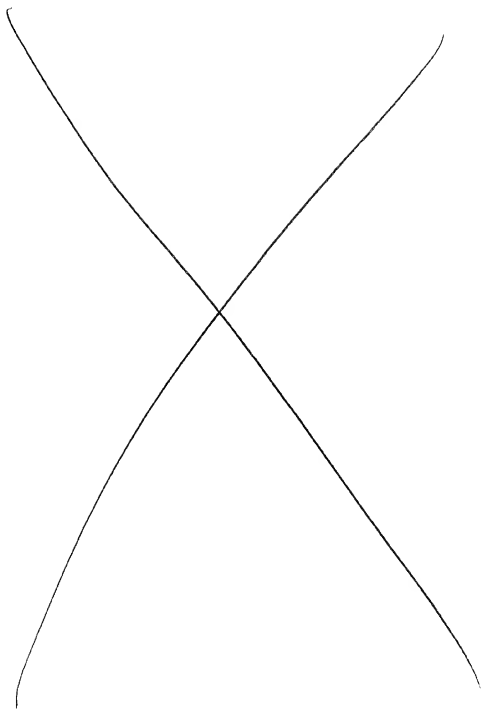
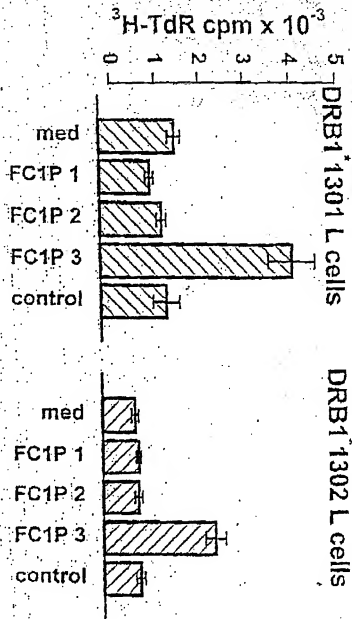
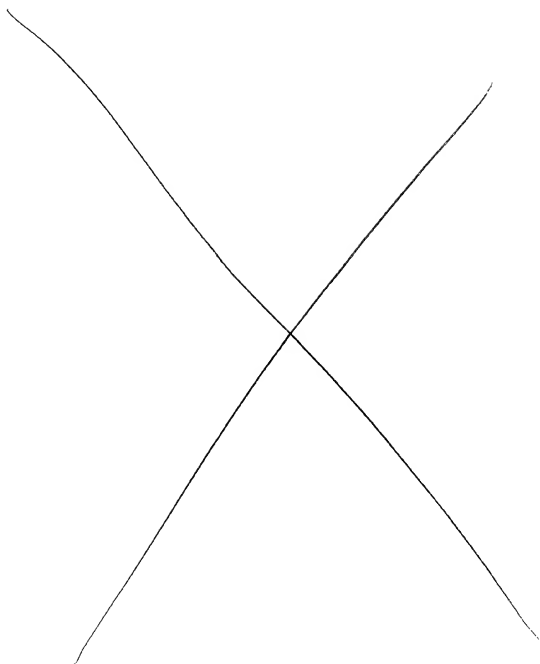


Figure 3





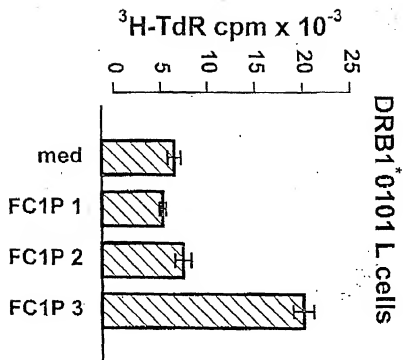


Figure 4

PCT/GE 04/000020

23/77 filed 13-1-99

Eric Peter Carlsson R.E.P.

